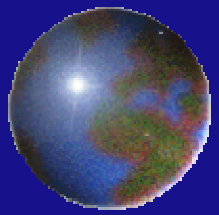
Vaccine Cell Substrates 2004



Introduction & Welcome

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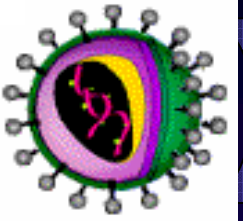
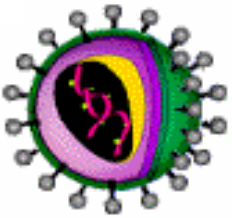
June 29, 2004



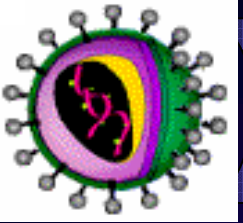
Welcome to VCS2004

- Welcome to Vaccine Cell Substrates 2004
- Thanks to our co-sponsor
  - International Association for Biologicals
- Thanks to our Scientific Steering Committee
- Thanks to our speakers and panel discussants
- Thank you for your participation
- We look forward to a productive conference



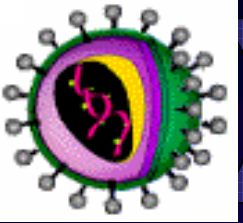
What is a cell substrate?

- The cells used to manufacture viral vaccines
- Vaccine cell substrates require characterization
- All viral vaccines are manufactured using a cell substrate



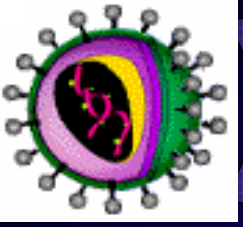
Why are cell substrates an issue?

- A horse named Jim
- SV40 contaminated early poliovirus vaccines
- Generally vaccines are given to HEALTHY people, most often INFANTS or children, to prevent a risk of POSSIBLE disease



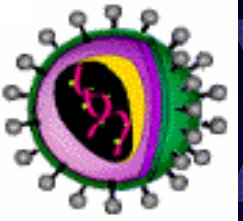
Therefore . . .

- Because of the risk/benefit considerations, vaccines are expected to be:
  - very safe
  - not to contain contaminants that would risk the health of otherwise healthy individuals



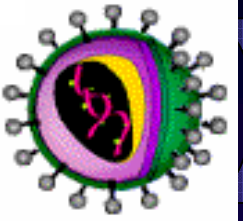
Early cell substrate policy

- Concern of CAUSING CANCER or other latent diseases
  - scientists knew that human leukemias occurred, but had not yet identified human leukemia viruses even though
  - animal leukemia viruses were known
- Thus, it was decided that only “normal” cells should be used as vaccine cell substrates
- What “normal” meant was not clarified
  - primary cells and tissues
  - secondary cultures
  - diploid cell strains, which senesce



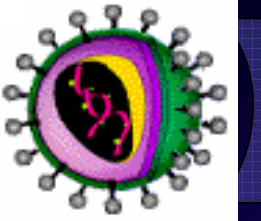
Evolution of policy

- **Over time, what makes an acceptable vaccine cell substrate has evolved**
  - **primary cells are no longer preferred**
  - **“normal” has evolved towards non-tumorigenic**
  - **immortalized, but non-tumorigenic cells are considered acceptable now**
  - **tumorigenic cells are being permitted to be used for investigational vaccines (and at least one licensed vaccine in Europe)**



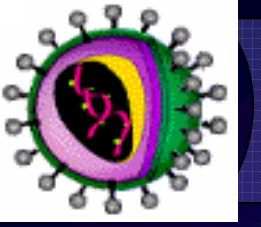
What has driven evolution?

- Much better understanding of molecular pathology of cancer - multi-step process
- Advances in state-of-the-art in manufacturing and testing
- Better methods for detecting oncogenic viruses
- Clinical experience with vaccines made from various cell substrates
- Need to develop vaccines for viruses that do not propagate in previously accepted substrates



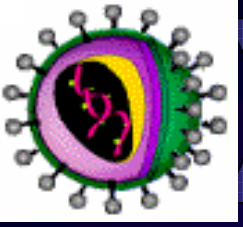
Cell Substrates for U.S. Licensed Vaccines

- "Simple" substrates - yeast
- Primary cells or animals
- Diploid Cells
  - MRC-5 - fetal human lung
  - WI-38 - fetal human lung
  - FRhL-2 - fetal rhesus lung
- Continuous Cell Line
  - Vero - African green monkey kidney (only U.S. licensed vaccines made in Vero cells are purified and inactivated – Inactivated Polio Vaccines)



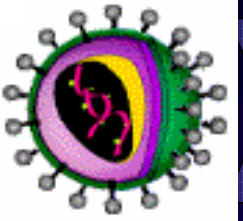
Cell Substrates for Investigational Vaccine Candidates

- In addition to the cell substrates for U.S. licensed vaccines . . .
- "Simple" substrates
- Insect Cell Lines
- Primary Cells
- Continuous Cell Lines
  - Vero
  - Transformed Cell Lines
  - Tumor-derived Cell Lines



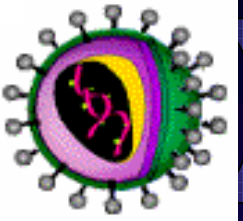
Potential sources of contaminants

- Cell Substrate used in vaccine production
- Viral Seed
- Cell Substrate(s) used in passage history of Viral seed
- Animal-derived or human-derived materials used in culture (passage history or production) or downstream processing (e.g., albumin added as virus stabilizer)
- Personnel
- Facility
- Other production materials (flasks, etc.)



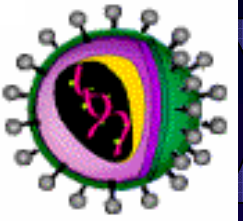
Safety Concerns for Cell Substrates

- Types of contamination
  - Adventitious Agents
  - Tumorigenic Potential
  - Residual Cellular DNA
    - infectivity
    - tumorigenic potential



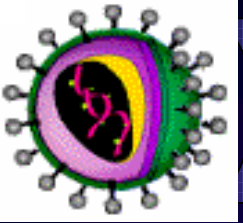
Adventitious Agents

- Adventitious agent tests, as will be described
- Concerns for novel substrates
  - expressed viruses
    - Contaminating adventitious or endogenous oncogenic viruses
    - recombinant viral elements
  - cellular DNA containing infectious proviruses or viral genomes



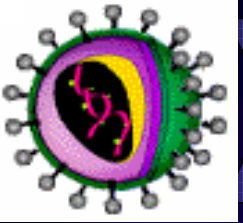
Tumorigenicity

- Potential sources of transmitting tumorigenic potential from cell substrate to final product (viral vaccine)
  - cells themselves
  - cellular proteins (oncoproteins, growth factors)
  - adventitious or endogenous (oncogenic) viruses
  - cellular DNA



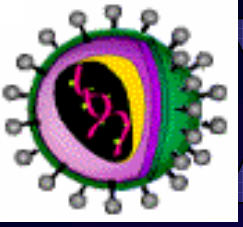
Tumorigenicity (2)

- **Cells**
  - even the least purified vaccines are generally filtered to remove potential bacterial contamination, which would also remove substrate cells
- **Cellular Proteins**
  - no way to replicate, so they would only be able to exert “effect” briefly, before being degraded in vaccinee



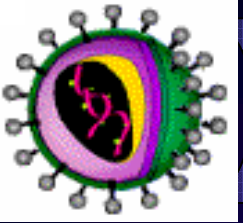
Tumorigenicity (3)

- Adventitious or Endogenous Oncogenic Viruses
  - difficulty in screening methods
  - unknown or occult agents
- Cellular DNA
  - activated oncogenes
  - insertional mutagenesis
  - oncogenic proviruses or viral sequences
  - methods for assessing tumorigenicity of DNA



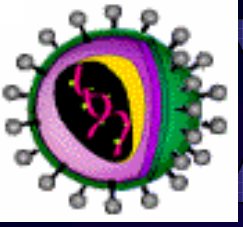
Cell Substrate Characterization

- Each manufacturer characterizes the cell substrates banked and used in production in their facility
  - history of isolation and banking
  - growth characteristics
  - karyology, identity, and tumorigenicity
  - freedom from adventitious agents



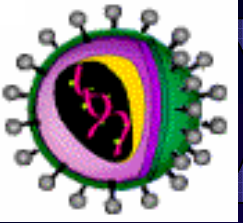
Tests to Characterize Cell Banks

- Karyology
- Isoenzyme analysis
- Tumorigenicity
  - tumor formation (progressing nodules and/or metastases) in immunosuppressed rodents
  - colony formation in soft agar
  - not necessary for cells of rodent origin (as all continuous cell lines of such are tumorigenic by in vivo assay)
  - other cells expected to pass tumorigenicity testing



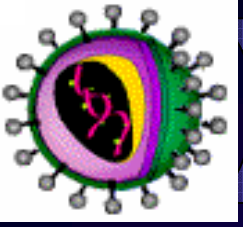
Adventitious Agent Tests

- Bacterial and fungal sterility (610.12)
- Mycoplasma (& spiroplasma)
- Mycobacteria (animals/culture-650.13)
- Viruses (*in vitro*, *in vivo*)
  - Acute (Lytic, Hemadsorbing/Hemagglutinating)
  - Latent (e.g., retroviruses or other oncogenic viruses)
- Retroviruses
- Specific tests (PCR)
- Animal-derived raw materials
  - 9 CFR 113 tests
  - from BSE-free countries



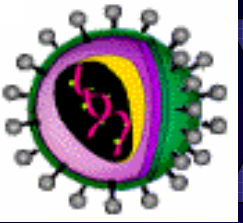
Purpose of Conference

- To inform the field of existing data and progress on addressing specific scientific issues related to cell substrates
- To discuss the continued validity of existing tests and appropriateness of new ones at this time
- To develop consensus recommendations for the field to address these issues
  - Implementing suggested recommendations
  - Identifying research gaps that preclude decision-making



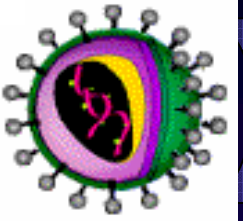
Topics for conference

- Oncogenicity of cellular components
  - Latent viruses
  - Cellular DNA
- Viral adventitious agent test methods
- Level of assurance provided by current tests
- Bovine viruses in bovine-derived raw materials (particularly serum)
- Bovine spongiform encephalopathy (BSE) agents as potential cell substrate contaminants
- Bonus session – testing performed on specific novel vaccine cell substrates



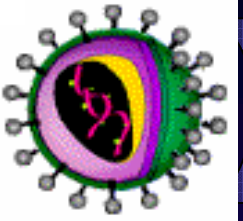
Questions for panel discussion

- **DNA oncogenicity:**
  - **Are there sufficient data to address whether or not cellular DNA can be oncogenic?**
  - **What sensitive and validated methods can be applied for cell substrate characterization, to address the potential of cellular DNA being oncogenic?**
  - **Are positive control(s) appropriate, in view of the absence of data to demonstrate that cellular DNA is oncogenic?**
  - **Can an alternative approach to testing, such as risk assessment, be used to address this issue?**



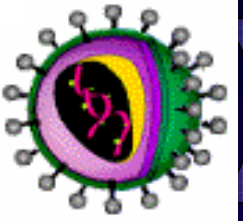
Questions for panel discussion (2)

- **Viral oncogenicity:**
  - **What methods can be implemented to best detect adventitious or endogenous oncogenic viruses or viral nucleic acids in a cell substrate?**
  - **What are the sensitivity and specificity of such methods?**
  - **Can in vitro methods alone be implemented?**



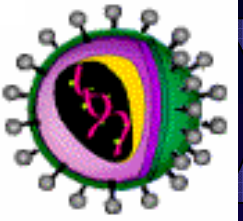
Questions for panel discussion (3)

- **Replacement of in vivo adventitious virus test methods:**
  - **Given sensitivity and breadth of in vitro adventitious virus test methods, can they serve to adequately replace part or all of the existing in vivo methods routinely used to characterize cell substrates?**
- **Level of assurance:**
  - **What amount of a cell bank should be tested in each characterization test?**
  - **What amount of a serum lot should be tested?**



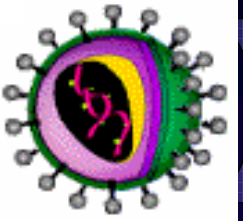
Questions for panel discussion (4)

- **Bovine (and porcine) viruses:**
  - **Which bovine viruses are likely to be found in serum?**
  - **Which of these can be amplified in cell culture, if inadequately documented serum was used in the passage history of a cell substrate or viral seed?**
  - **Which are potentially pathogenic for humans?**



Questions for panel discussion (5)

- **BSE:**
  - Can the BSE agent propagate in cell culture?
  - Can it be passaged into vaccine products from vaccine cell substrates?
  - What assay methods are available or in development and are adequate (sensitive and validated) for screening cell substrates and raw materials used in vaccine production?
  - When there is uncertainty in raw material traceability for a seed material or cell substrate's passage history, and a risk assessment is performed, what degree of residual risk is acceptable?



Acknowledgements

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